ORIGINAL PAPER

TRAUMA AND RECONSTRUCTIVE UROLOGY

Use of an acellular collagen-elastin matrix to support bladder regeneration in a porcine model of peritoneocystoplasty

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Introduction Bladder reconstruction without using the intestine remains a challenge to this day despite the development of new biomaterials and cell cultures. Human bladder engineering is merely anecdotic, and mostly in vitro and animal studies have been conducted.

Material and methods In our study using a porcine model, we performed a bladder augmentation using an autologous parietal peritoneum graft (peritoneocystoplasty) and determined whether the attachment of an acellular collagen-elastin matrix (Group 1) or lack of (Group 2) had better histologic and functional results. Thus far, peritoneocystoplasty has rarely been performed or combined with a biomaterial.

Results After 6 weeks, we observed different degrees of retraction of the new bladder wall in both groups, although the retraction was lower and the histological analysis showed more signs of regeneration (neoangiogenesis and less fibrosis) in Group 1 than when compared with Group 2. No transitional cells were found in the new bladder wall in any of the groups, and no differences were observed in the functional test results.

Conclusions Performing a peritoneocystoplasty is an easy and safe procedure. The data supports the benefit of an acellular collagen–elastin matrix to reinforce bladder regeneration. However, in our study we observed too much retraction of the new wall and the histologic results were not acceptable to consider it an appropriate cystoplasty technique.

Key Words: acellular matrix ‹› bladder regeneration ‹› biomaterial ‹› peritoneocystoplasty ‹› tissue engineering

INTRODUCTION

Bladder reconstructive surgery encompasses surgical procedures involving the substitution or enlarging of the bladder. Radical cystoprostatectomy due to muscle-invasive bladder cancer is the most prevalent condition warranting the removal and substitution of the urinary bladder [1]. Augmentation cystoplasty is required in certain types of neurogenic bladder dysfunction, interstitial cystitis (painful bladder syndrome), and severe radiation- induced or urogenital tuberculosis contracted bladder. Currently, the human intestine is used in daily clinical practice to substitute or enlarge the bladder. It has many drawbacks, not only due to bowel resection, but also to relevant complications related to the contact of the urine with a tissue not biologically prepared for this purpose, which involves an increase in morbidity rates [2, 3]. Bladder tissue engineering is dedicated to design and produce a material that can replace the bladder tissue instead of using the intestine. Mostly in vitro and animal studies have been

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conducted with different types of biomaterials, cell cultures and growth factors [4]. However, the human bladder engineering is merely anecdotic and remains an enormous challenge to this day [5, 6, 7]. The ideal material should be readily biodegradable, biocompatible and useful as a scaffold, so that it could host tissue development [8, 9].

In our study using a porcine model, we made bladder augmentations using an autologous parietal peritoneum graft (peritoneocystoplasty) and determined whether the attachment or not of an acellular collagen–elastin matrix to this peritoneum graft had better histologic and functional results. Thus far, peritoneocystoplasty has rarely been performed or combined with a biomaterial.

MATERIAL AND METHODS

The animals were divided in two groups: Group 1 was peritoneocystoplasty plus biomaterial and Group 2 was peritoneocystoplasty without the biomaterial. The total survival time of the animals was six weeks.

Animals and ethics statement

Experiments were performed on 16 female hybrid pigs (Large White x Landrace) weighing 27–40 kg. All procedures were approved by the Animal Experimentation Ethics Committee (registration number: 20/13) of the Vall d'Hebron Research Institute and conducted in compliance with the Spanish legislation in accordance with the European Union directives (2010/63/EU). The experiment was categorized as a stage 0 (preclinical study) for the evaluation of a new medical product according to IDEAL-Device (Idea, Development, Evaluation, Assessment and Long-term) [10].

Graft preparation

The biomaterial consists of a 1 mm-thick structurally intact native collagen matrix coated with α-elastin hydrolysate of bovine origin (Matriderm®, MedSkin Solution Dr. Suwelack AG, Germany). It is sterilized by gamma irradiation after freeze-drying and is stored at room temperature. It has been tested in clinical trials to treat full-thickness burns, trauma and reconstructive wounds. It has shown good results when applied simultaneously with split-thickness skin grafts in single-stage operative procedures. The material promotes cell migration and proliferation and encourages early neoangiogenesis [11, 12]. We also used a parietal peritoneum graft obtained through a midline down laparotomy. A 10×10 cm graft was completely dissected from the anterior abdominal wall musculature. The graft was later taken folded in half, and then folded in half again. It was stored in a covered container with a 0.9% sterile saline solution at room temperature.

Anesthesia and surgical procedure

A urinary catheter was inserted before initiating the surgical procedure to perform the first functional test (as explained in the functional test section). A lower midline laparotomy incision was performed to access the peritoneal cavity. After a general inspection, we completely dissected the peritoneum graft from the anterior abdominal wall musculature and stored it. Afterwards, we located the bladder and performed a 5×5 cm exeresis of the anterior wall. Afterwards, we made simple interrupted stitches of polypropylene (Prolene®, Ethicon) around the edges of the exeresis (in the serous membrane) as reference points. Six weeks later, these marks helped us to identify the surgical borders and measure macroscopically the eventual graft shrinkage. After performing these referral stitches, we randomly divided the pigs in two groups. In Group 1, we substituted the resected bladder wall with a 5×5 cm compound graft of peritoneum plus the biomaterial. Both were attached easily by contact in the operating theater without glue or stitches. The biomaterial was in the inner part of the bladder and the peritoneum was in the outer part. The graft was sutured to the bladder wall (all layers) with two running stitches of poliglecaprone 4-0 (Monocryl®, Ethicon). In Group 2, we performed the same procedure, but only with the peritoneum graft without attaching the biomaterial. The final macroscopic results are shown in Figure 1. The animals kept the urinary catheter for 10 days. After six weeks, we repeated the functional test and performed the cystectomy and euthanasia. The animals were euthanized by receiving an anesthetic IV overdose of 2 g of tiobarbital (Tiopental®, B. Braun Melsungen AG).

Specimen harvest and histological procedures

The gross examination analysis of the bladder, subsequent to euthanasia, involved four steps: identify the new bladder wall formed from the graft, study the seroma formation, local infection, and tissue integrity. In measuring the new bladder wall, the polypropylene stitches previously placed along the edges of the resected area were very useful. We obtained this measure in centimeters and calculated the eventual shrinkage rate of the new wall. We used qualitative scales to evaluate the rest of the parameters. First, the seroma in the new wall was classified into A (no alteration) or B (mild or massive seroma).

Second, we evaluated whether signs of infection were present and categorized the signs as either A (no alteration) or B (purulent exudate). Lastly, we carefully examined the perimeter of the graft to study the tissue integration with the normal bladder wall and classified it as either A (complete integration) or B (narrow areas at the edges or lack of integration). The histological analysis was performed by selecting six representative sections of the bladder (new wall, transition zones and normal wall). These tissue sections were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 4 μm. These sections were stained using the hematoxylin and eosin (H&E), Gömöri trichrome and Elastica van Gieson. The slides were qualitatively assessed for cellular organization with regard to the vessel density, cellular infiltration, fibrosis, presence of urothelial cells and traces of the biomaterial. The quantification of fibrosis and vascular rate was counted on a light microscope at 40x magnification field (4x objective and 10x ocular). To quantify the fibrosis, we used an histoscore (H-score) that was calculated by assessing the percentage of fibrosis cells and the intensity of the Gömöri staining (graded as 0: non-staining; 1+: week; 2+: moderate; 3+: strong). The final score was calculated [1x(% cells 1+) + 2x (% cells 2+) + 3x(% cells 3+)], and it ranged from 0 to 300. The blood vessels were quantified by averaging the total number in 10 different microscopic fields of the new bladder wall per animal.

Functional test

A volume/pressure test by filling the bladder to the leak point was performed just before the first surgery and before euthanasia. We used an 18 Ch double-lumen urinary catheter connected to a custom-designed software, and we filled the bladder with normal saline at 25 ml/min. We set the maximum bladder capacity when the pericatheter leak was detected. Note that the functional test before euthanasia was made after the laparotomy, so that we could observe how the bladder was expanding throughout the filling process, especially in the graft area.

Statistical analysis

Total number of animals was calculated after the preliminary results in relation to the retraction rate (we presumed a difference of 60% between Group 1 and 2). With a power (1- β-risk) of 0.8 and α-risk of 0.05, we needed 7 animals per group to find significant statistical differences. Univariate analysis was used to describe the animal weight, retraction, differences in maximum volume and pressure, H-score and number of vessels. In order to describe the variables we calculated the mean, median, variance and standard deviation. Comparisons between Group 1 and Group 2 were analyzed with the Mann-Whitney U test using SPSS® (V24.0). Statistically significant values were defined as $p < 0.05$.

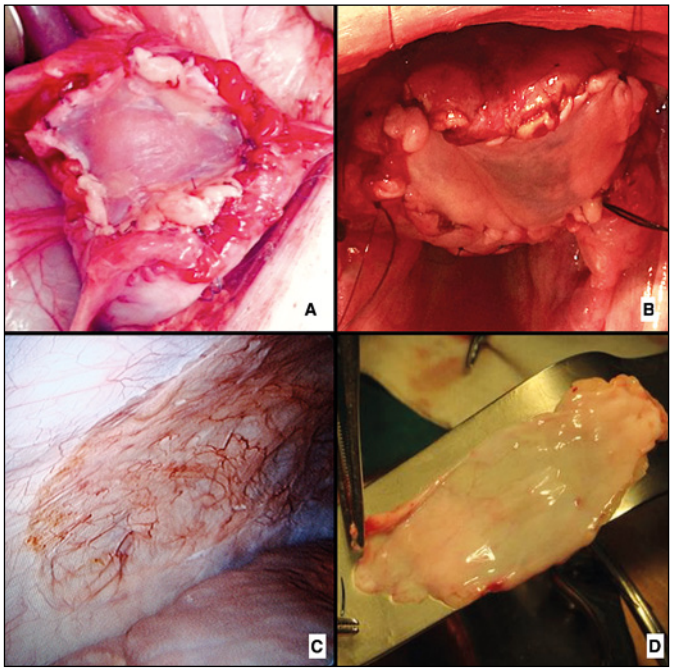

Figure 1. *Representative macroscopic steps of the implantation procedure.* **A.** *Peritoneum + biomaterial cystoplasty.* **B.** *Peritoneum cystoplasty.* **C.** *Anterior abdominal wall after removing the peritoneum graft.* **D.** *Peritoneum graft.*

RESULTS

General considerations and complications of the surgical procedure

The original idea of dividing the animals into two exact groups was not possible in three cases because of the fragility of the peritoneum graft. We could not perform adequate stitches because they provoked ruptures at the graft edges. In these three cases, we added the biomaterial to strengthen the peritoneum graft. Thus, we used 11 peritoneum plus biomaterial grafts (Group 1) and 5 peritoneum grafts (Group 2) in total. As a serious complication, one animal (Group 1) required further surgery because of an intraperitoneal urinary leakage caused by the partial rupture of the graft suture line, which was successfully repaired. We noticed the importance of cutting the tip of the urinary catheter (respecting the balloon) to prevent graft damage. All the animals were weighed twice: at the beginning and after six weeks. Results are shown in Table 1.

Macroscopic examination

We did not observe seroma, local infection, or lack of integration in any of the groups after six weeks. In two cases belonging to Group 1, a 1×1 cm stone attached to the new bladder wall was observed (Figure 2D). The mean retraction in Group 1 was 28.6% (10–80%) and that in Group 2 was 72% (60–80%), which was statistically significant (p = 0.007). Macroscopically, that difference was self-evident, as shown in Figure 2. In all cases we observed a correct wall thickness without narrow areas (Figure 3).

Histological analysis

In Group 1, the H&E stain examination (Figure 4) of the new-born bladder wall tissue at six weeks showed mucous ulceration, moderate fibrosis tissue, glandular cystic cystitis, poor neovascularization and hardly any bundle of disorganized smooth muscle tissue. No urothelial cells were observed in the new bladder wall, although we observed emerging signs of urothelial epithelization and chronic inflammatory eosinophilic response at the edges. Most of the new vessels were found to be subepithelial, and fibrosis was predominantly found in the deepest part of the tissue (Figure 5). In addition, traces of the monocryl stitches were found, but there were hardly any traces of the biomaterial. In Group 2, the H&E stain showed mucous ulceration, massive fibrosis tissue and lack of urothelium and muscle tissue. Using the Gömöri trichrome stain, the mean H-score assessment of fibrosis was 40.9 (10–65) for Group 1 and 129 (50–220) for Group 2, and the difference was statistically significant (p = 0.007). To quantify the vessels of the new wall, we used the H&E and the Elastica van Gieson stain. The average number of blood vessels per field in Group 1 was 61.3 (12–103) and 33.8 (17–52) in Group 2, and the difference was also statistically significant (p = 0.03).

Functional results

The test was finally conducted on 9 animals (Group 1: 6, Group 2: 3) because placing the urinary catheter in the the remaining animals was not possible because of urethral anatomical limitations. The mean pressure variation in Group 1 was 3.5 mmHg and that in Group 2 was −0.3 mmHg, which was not statistically significant (p = 0.051). Results are shown in Table 1.

Table 1. *Results of the variables investigated and comparison between groups*

Variable*	Group 1	Group 2	p-value
Weight increase, kg	5.9 ±2.9	8.2 ±3	0.18
Retraction rate, %	28.6 ±19.5	72 ±8.3	<0.05
Fibrosis H-score	40.9 ±18.2	129 ±64	<0.05
Vessels, **	61.3 ±24.8	33.8 ±13.6	<0.05
Volume variation, mL	30 ±103.7	-46.6 ±61.1	0.16
Pressure variation, mmHg	3.5 ±2.3	-0.3 ±2	0.051

* Values are reported as mean ± standard deviation
** Average number of blood vessels per field (40x magnification)

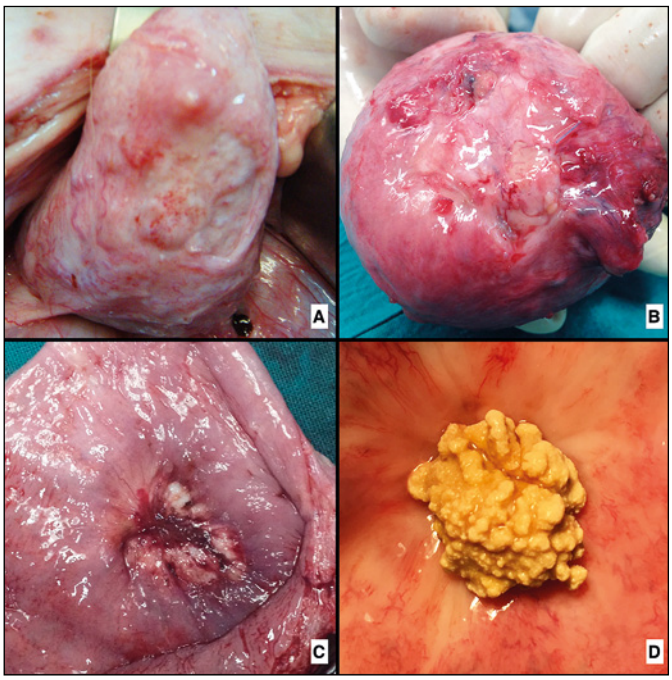

Figure 2. *Macroscopic overview 6 weeks post-surgery.* **A, B.** *Mild retraction in Group 1. See the polypropylene marks in Figure 2B.* **C.** *Major retraction in Group 2.* **D.** *Stone attached to the new bladder wall in Group 1.*

DISCUSSION

Obtaining the best technique and material to perform an augmentation cystoplasty or a bladder substitution without using the intestine remains a challenge [13, 14]. Nowadays, they can only be offered within a clinical trial setting. Since the announcement of the in vitro urinary bladder reconstruction by Atala et al., interest in bladder tissue engineering has increased [15]. To date, only 18 studies have been conducted on humans (approximately 169 patients) using biomaterials and all the other studies have been performed using animal models or based on in vitro tests [6, 16]. The different biomaterials can be classified into naturally derived scaffolds (collagen, alginate, elastin, fibronectin, and hyaluronic acid), acellular tissue matrices (bladder acellular matrix, small intestinal submucosa, dermis, gallbladder, and amniotic membrane), and synthetic scaffolds (silk-

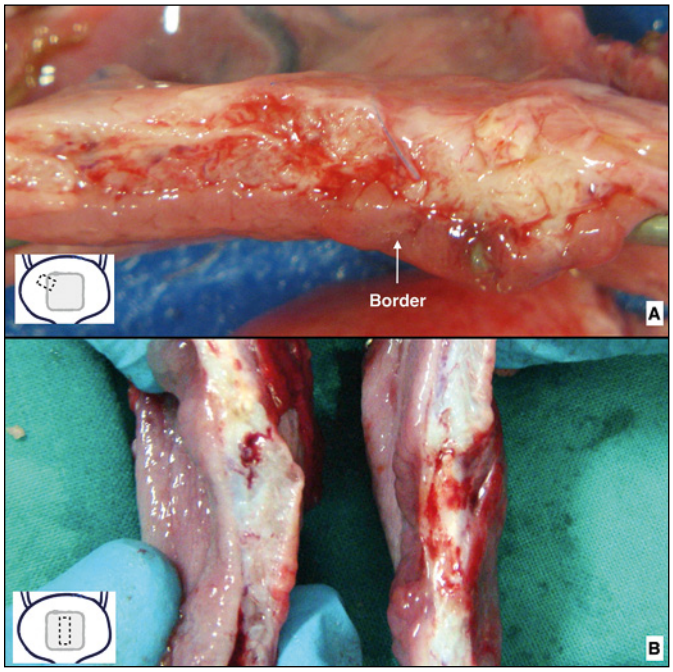

Figure 3. *Representative samples of the bladder wall thickness in Group 1.* **A.** *Transition area between normal bladder and new wall, with the polypropylene marks.* **B.** *Mid-sagittal plane of the new wall.*

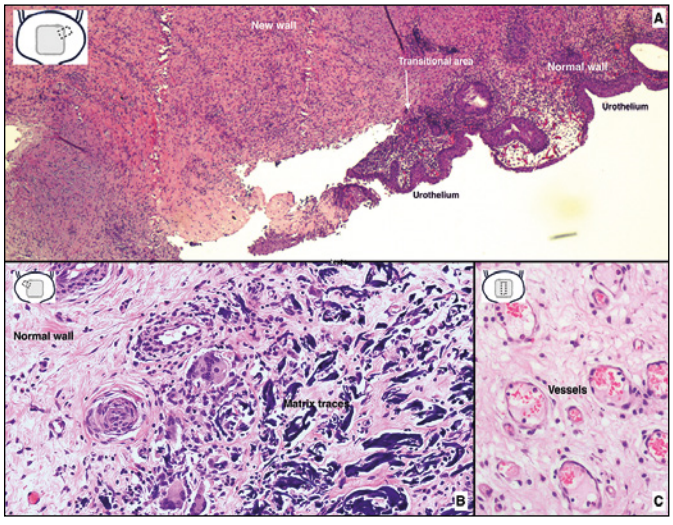

Figure 4. *Representative histological overview (hematoxylin and eosin) after 6 weeks in Group 1.* **A.** *Transition area. Urothelial cells are only present in the normal bladder (right part).* **B.** *Inflammatory response (left), matrix traces (right).* **C.** *Vessels in the new wall.*

based materials and different types of poly-α-esters as polyglycolic acid) [17]. There are studies that have incorporated exogenous growth factors to the biomaterial in order to promote better results in terms of cellular repopulation and neovascularization.

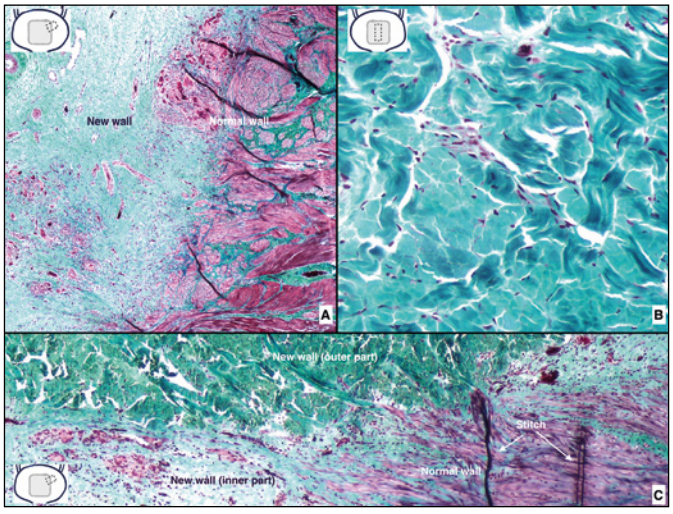

Figure 5. *Representative histological overview (Gömöri trichrome) after 6 weeks.* **A.** *Transition area in Group 1. See muscle fibers on the righthand side trying to emerge to the new wall (left part).* **B.** *Fibrosis (H-score 3+) in Group 2.* **C.** *Predominantly the newly born tissue was in the inner part and the fibrosis was in the outer part, in Group 1.*

Incorporating these factors could be debatable, since sometimes their uniform distribution cannot be assured and high doses could affect the healing process [8, 18]. In general, experimental models conducted are complex and they usually require autologous or stem cell cultures and laboratory procedures that are difficult to perform in daily clinical practice. Furthermore, a well-stablished previous plan is always needed, and thus the use of biomaterials, cell cultures and exogenous growth factors cannot be decided upon the perioperative period (e.g., if the surgeon considers that the intestine cannot be used). Our main reason to perform peritoneocystoplasty with a parietal peritoneum graft was due to the relative ease of the technique and its reproducibility, the capability to do it without cell cultures and other in vitro procedures, and its limited experience as a bladder substitute tissue and in combination with a biomaterial. In 1988, Youssef et al. performed bladder augmentations in rats using a peritoneal graft with a polyglycolic acid mesh [19]. After the first month, the histological analysis showed a transitional epithelium and muscle fibers on the new wall. In 1990, Weingarten et al. performed myoperitoneocystoplasties in six ferrets reporting a median increase of 67% of the bladder capacity, transitional epithelium on the new wall, and no complications [20]. Close et al. published a group of 10 peritoneocystoplasty procedures in a sheep model with poor results. The bladder capacity did not increase, and the histological analysis showed an extensive fibrosis of the new wall [21]. In humans, two

similar studies (21 patients in total) used a peritoneal flap to cover a bladder auto-augmentation [22, 23]. After detrusorrhaphy, a segment of the peritoneum was harvested by making an inverted U incision and tacking it to the bladder to promote the expansion of the exposed urothelium. The results were not satisfactory in functional terms. Our reasons for using an acellular collagen–elastin matrix were its availability in the burn unit of our hospital and the lack of experience in experimental bladder surgery with this type of biomaterial and peritoneum. This matrix acts as a scaffold to promote bladder regeneration, it is biodegradable and easy to handle, and does not induce an immune response. In general, it has better results in urothelial epithelialization rather than in muscle fiber regeneration, therefore it can finally affect the functional outcomes [24].

Our results in a pig model showed that performing a peritoneocystoplasty using the parietal peritoneum graft was not a good choice because the new wall was contracted and was mainly composed of fibrotic tissue. However, when we added an acellular collagen–elastin matrix to the peritoneum graft, the results were better because the retraction rate was lower and the histological analysis showed more signs of regeneration (greater number of vessels and less fibrosis). Nevertheless, in that last group the retraction was also high, and we found a urinary stone attached to the new bladder wall (inner part) in two animals. We did not observe transitional cells on the new wall in any of the groups. We believe that this outcome was probably due to the limited survival time according to other studies [8, 24, 25]. We want to emphasize that in bladder regeneration, not only is the presence of urothelial cells important, but also the muscle fiber regeneration, which we did not properly observe in any of the groups. Previous studies on animals and humans showed good results in terms of urothelial re-epithelialization, but with an inadequate muscle regeneration because it was incomplete, disorganized, not well integrated into the new wall, and without adequate vascularization or innervation [7]. This fact determines the final bladder functionality [24, 26]. Some studies have even performed a specific mechanical analysis to study the tensile strength of the new wall [27, 28]. In terms of the high rate of the biomaterial degradation after six weeks, we obtained the same results according to previous experiments on bladder and other organs [29]. The functional results did not significantly change between both groups. We believe that the bladder possibly balanced the structural changes and maintained the same maximum volume and pressure. However, our study has its limitations. First, we point out the complex handling of the peritoneum graft that forced us to add the biomaterial in some of the animals. This issue was the reason why both groups were not equal. Perhaps we should have increased the number of animals to strengthen the statistical results. Second, we believe that the survival time of the animals should have been longer in order to promote a better regeneration of the new wall. Third, in order to support the final histological results, we should have added an immunohistochemical staining to study inflammatory response markers or structural proteins (e.g. antibodies against smoothelin, α-actin, desmin, pancytokeratin, uroplakin III, etc.) [8, 28, 30, 31]. Finally, in some cases we faced urethral anatomical limitations, so the functional test could not be properly performed in all animals.

CONCLUSIONS

Currently, the clinical application of biomaterials for performing a regular bladder reconstruction is not immediately forthcoming and experimental methods are usually difficult to apply in daily clinical practice. Performing a peritoneocystoplasty is an easy and safe procedure, uses an autologous graft and does not require any preparation. However, at 6 weeks we observed too much retraction of the new wall. We consider that the histologic results were not good enough although the group with the biomaterial had better outcomes. In the future, further experiments using peritoneum grafts could possibly be conducted but using different biomaterials, growth factors and/or cell cultures. However, replacing the intestine as the gold standard tissue for bladder reconstruction remains a great challenge.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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